Synthesis and Characterization of Copper Nanoparticles by Chemical Method Using Ascorbic acid as a Reducing Agent in Presence of Metronidazole Benzoate (MTZ.B) (1-(2-benzyloxy ethyl)-5-nitro methyl imidazole) Drug

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Abstract

Copper nanoparticles (Cu NPs) have synthesized via reduction of copper (II) sulfate pentahydrate salt (Cu SO$_4$ .5H$_2$O) using ascorbic acid as reducing and polyvinyl pyrrolidone PVP (M.Wt 10,000) as a stabilizing agents in presence of Metronidazole Benzoate (MTZ.B) (1-(2-benzyloxy ethyl)-5-nitro methyl imidazole) drug by chemical method. The resulting Cu NPs were characterized by "UV–Vis spectroscopy, atomic force microscopy (AFM), X-ray diffraction (XRD) and FT-IR spectroscopy". Keywords: Copper nanoparticles, Surface Plasmon resonance (SPR), X-ray diffraction (XRD), UV–Vis spectrophotometry.

Introduction

Copper exhibit considerable biochemical action either as an essential trace metal or as a constituent of various exogenously administered compound in human [1]. Copper nanoparticles of high surface to volume ratio, are very reactive as antimicrobial agents as they interact closely with microbial membranes [2, 3].

They have been reported to decrease the microorganism concentration by 99.9%. Copper nanoparticles supported on a matrix can be used as a bactericide agent to coat hospital equipment [2]. Cu nanoparticles have currently attracted a significant research attention owing to their widespread applications in powder metallurgical materials and electronic circuits, "such as lubricants, electronic materials, catalysts, thermal transfer, nano fluid, and optical devices" [4, 5].

Metronidazole Benzoate (MTZ.B) is chemically 1-(2-benzyloxy ethyl)-5-nitro methyl imidazole) or "2-(2-methyl-5-nitro-imidazol-1-yl) ethyl benzoate", one of the nitroimidazole derivative [6, 7, 8]. Metronidazole Benzoate(MTZ.B) has an extremely broad spectrum of protozoal and antimicrobial activity which can be used to clinical
advantage and also displays antibacterial activity against all anaerobic cocci and both anaerobic "gram-negative bacilli and anaerobic sporeforming gram-positive bacilli". It is clinically effective in trichomoniasis, amebiasis and giardiasis [9], as well as in a variety of infections caused by obligate anaerobic bacteria [7, 10]. Figure (1) shows the structures of (MTZ.B).

![Figure 1- Structures of Metronidazole Benzoate (MTZ.B)](image)

This work investigates the synthesis and characterization of copper nanoparticles (Cu NPs) using ascorbic acid as reducing and polyvinyl pyrrolidone PVP (M.Wt nearly 10,000) as a stabilizing agent in presence of Metronidazole Benzoate (MTZ.B) drug conjugates.

**Materials and Methods**

**Chemicals**

All the following chemicals in this study were used as received from suppliers: Metronidazole Benzoate (MTZ.B) (99.98%) (India), Copper (II) sulfate pentahydrate salt (CuSO₄.5H₂O) 98.5% (G.P.R.), zinc powder (99%) (Fluka), L-ascorbic acid (C₆H₈O₆) 99.7% (SCR, China). Polyvinylpyrrolidone (PVP) (M. Wt nearly 10,000) (C₆H₉NO)n 99% (Fluka), Hydrochloric acid (HCl) Analar (BDH).

**Instruments**

The absorption spectra in the "UV-visible region 200-1100 nm" were recorded on a SHIMADZE 1800 Double Beam "UV-Vis spectrophotometer". Fourier Transform Infrared (FT-IR) spectra were recorded on "Shimadzu FT-IR 8400S Fourier transforms", within the wavenumber region between 4000 and 200 cm⁻¹ using KBr and CsI discs, "Department of chemistry / College of Science / University of Baghdad". AFM images were obtained using AFM model CSPM "Scanning Probe Microscope / Department of chemistry/College of Science/ University of
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Baghdad", Samples were prepared by applying few drops solutions on a glass slide and dried under vacuum or by inert gas. And "XRD measurements were performed using a Shimadzu XRD-6000 X-ray diffraction spectrometer / Ministry of Science and Technology".

**Preparation of Antibiotic (MTZ.B) solution**

An aqueous solution of (MTZ.B) (3.6 x10^-4 M) was freshly prepared by dissolving 0.1 g of metronidazole benzoate powder in "100 mL" distilled deionized water DDW in "100 mL" volumetric flask.

**Synthesis of Copper NPs**

1- **Preparation of copper nanoparticles using ascorbic acid as a reducing agent**

Copper nanoparticles were prepared by following the method reported by Qingming , et al [11], with a slight modification concerning the concentration of the reducing agent, heating temperature and degassing time. An aqueous solution (33 mL) containing a dissolved mixture of CuSO4 · 5H2O (1.664 g) and polyvinyl pyrrolidone PVP (M.Wt nearly 10,000) (5 g) was put in " three- neck round- bottom flask " and reflux condenser, separatory funnel and gas inlet. The mixture was purged with nitrogen for 30 minutes and heated in a water bath at 70-80°C for 2 hours with continuous stirring. Then a solution of ascorbic acid (2.34g) in DDW (13 mL) was added in portions for 30 minutes under nitrogen atmosphere. The final concentrations of the three reactants were (0.2M, 10.8 x 10^-3 M, and 0.289 M, respectively) and the pH of solution was 7. The color changed from blue to orange to reddish brown, finally to red within 15 min of ascorbic acid addition Figure (2 a, b, c), which confirmed Cu NPs. Reaction was continued for 2h under nitrogen atmosphere. The solution mixture was measured in the UV-Visible region. The products were separated by centrifugation, washed several times with distilled water and ethanol, and finally dried with N2 gas and put in a test tube under N2 gas, (yield 0.1912 g).

2- **Synthesis of copper nanoparticles in presence of MTZ.B**

The preparation of MTZ.B / Cu NPs was performed following the same process as in section 1 using the same concentration of CuSO4 · 5H2O, polyvinyl pyrrolidone (PVP) and ascorbic acid (0.2M, 10.8 x 10^-3 M, and 0.289M respectively) , the same heating temperature range (70-80°C ) , at pH 7. After 1h of reaction, an aqueous solution of MTZ.B (3.6 x10^-4 M,10 mL) was added , 1 h after synthesis of Cu NPs under continuous stream of nitrogen gas with vigorous stirring .The reaction was continued for further 1h for completion, Figure (2 d).
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Results and Discussion:
Characterization of copper nanoparticles
Detection of copper nanoparticles by UV-visible spectrophotometry

The UV-Vis spectrophotometry have proved to be a very useful technique for studying some metal nanoparticles because the peak positions and shapes are sensitive to particle size. The "surface plasmon peak of copper nanoparticles" has been reported to be appearing at around "570 - 575 nm", in the range 20-80 nm [4, 12, 13, 14]. Dispersed copper nanoparticles were obtained through the reduction of Cu$^{2+}$ (0.2M) into Cu$^{0}$ using L-ascorbic acid (0.289 M) as reducing agent and pvp (10.8 x 10$^{-3}$ M) as a stabilizing agent. "L-ascorbic acid is a highly water-soluble compound with strong polarity. It behaves as a vinlylogous carboxylic acid in which the electrons in the double bond, hydroxyl group lone pair, and the lactone ring carbonyl double bond form a conjugated system. As such, the structure of L-ascorbic acid gives enough reducibility to convert Cu$^{2+}$ ions into Cu$^{(0)}$ nanoparticles", the possible mechanism for the formation of Cu nanoparticles is shown in Figure (3) as was suggested by Xiong, et al [4]. Ascorbic acid has been used as both reducing agent and antioxidant to prevent the reversible oxidation of Cu NPs. The reaction was carried out under nitrogen atmosphere to prevent the oxidation of Cu to Cu$^{+2}$ ions also.

Figure 1- The images show the solution of a- (copper (II) sulphate solution and pvp), b - After addition ascorbic acid , c - Synthesized copper nanoparticles after 15 min of ascorbic acid addition, d - Synthesized copper nanoparticles in presence of MTZ.B after 1 h.
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Figure 3 - The possible mechanism for the formation of Cu nanoparticles. The UV–Visible spectra of the single reactants and the resulting solution mixtures in aqueous solution were measured in the range $\lambda$ 200-1100 nm.

Figure (4 a ) shows the spectra of CuSO$_4$.5H$_2$O, L-ascorbic acid and copper nanoparticles prepared from the reaction mixture (copper (II) sulphate salt solution, pvp, and ascorbic acid). The final concentrations of the three reactants were (0.2M, 10.8 x $10^{-3}$ M, and 0.289 M) respectively, under nitrogen atmosphere and the pH of solution was 7, PVP (M.w10 000) is used as dispersing agent, the measured pH of solution was 7. The spectrum of CuSO$_4$ in aqueous solution displayed a broad band at $\lambda$ 807 nm assigned to the" $^2$Eg→$^2$T$_{2g}$" transitions of distorted octahedral Cu (II) complex [15, 16]. Spectrum of L-ascorbic acid displayed high intensity band at $\lambda$ 253 which was attributed to $\pi→\pi^*$ transitions [17, 18]. The spectrum of copper nanoparticles displayed a band at $\lambda$ 575 nm refers to the "surface plasmon resonance of copper nanoparticles" with particle size 20-80 nm [4, 12, 13, 14].
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Figure 4 a - The UV-Visible spectra of CuSO$_4$.5H$_2$O (2x10$^{-2}$ M), L-ascorbic acid, Cu NPs and their images in aqueous solution using (copper (II) sulphate solution, pvp (Mw nearly 10 000), and ascorbic acid (0.2M, 10.8 x 10$^{-3}$ M, and 0.289M, respectively) at pH 7 after 30 minutes.

The spectrum of (MTZ.B) Figure (4 b) displayed two absorption bands maxima at $\lambda$ 232 and 319 nm which was attributed to $\pi$-$\pi^*$ transitions of imidazole ring [19, 20]. The spectrum of MZ /Cu NPs colloid exhibited bathochromic shifts of the ligand bands which appeared at 235 and 324 nm and the appearance of an absorption $\lambda$ 573 n m corresponding SPR of Cu NPs [4, 12, 13, 14]. The colloid was stable with preservation of color of Cu NPs for more than 3h then the red color changed to black as a result of oxidation by atmospheric oxygen.
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Figure 4 b - The UV-Visible spectra of antibiotic MTZ.B (3.6 ×10⁻⁴ M) and MTZ.B Conjugation Cu NPs in aqueous solutions at room temperature using (copper (II) sulphate salt solution), pvp, ascorbic acid and MTZ.B (0.2 , 10.8 x 10⁻³, 0.289 and 3.6 x 10⁻⁴ M respectively) at pH 7 after 1 h.

X – Ray diffraction (XRD)

The "XRD" patern of pvp-protected copper nanoparticles synthesized by ascorbic acid (0.289M) as a reducing agent is shown in Figure (5). Peaks are very sharp due to the high nanocrystalline nature of copper nanoparticles. Three diffraction peaks were observed at 2θ = (43.47, 50.59 and 74.26) degrees corresponding to the" planes [111], [200] and [220] of face centred cubic (fcc) Cu metal "crystal lattice [21, 22, 23].
Average particle size has been estimated by using Debye-Scherrer formula equation [24, 25].

\[ D = \frac{k \cdot \lambda}{\beta \cos \theta} \]

where ‘D’ is particle diameter size.
"θ is the Bragg angle".
"λ is the wavelength of the X ray" used (0.154 nm).
"β is the breadth of the pure diffraction profile in radians on 2θ scale".
"K is a constant approximately equal to 0.90 and related both to the crystalline shape and to the way in which θ is defined". "β is the Full Width at Half Maximum (FWHM) values measured for [111], [200] and [220] planes of reflection were used with the Debye-Scherrer equation to calculate the size of the nanoparticles". "The value of D calculated from the [111], [200] and [220] reflection was (42.31) nm. The value of D calculated from the [111] reflection was (46.33) nm".
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Figure 5 - The XRD pattern of the pvp-protected copper nanoparticles prepared at pH 7 using ascorbic acid (0.289M) as a reducing agent.

The XRD pattern of the Cu NPs in presence of (MTZ.B) is shown in Figur (6). Three diffraction peaks were observed at 2θ = (43.67, 50.79 and 74.43) degrees corresponding to the planes [111], [200] and [220] of face centred cubic (fcc) Cu metal crystal lattice, respectively [21, 22, 23]. The value of "D calculated from the [111], [200] and [220] reflection " was (40.25) nm. The value of" D calculated from the [111] reflection " was (44.67) nm.

Figure 6 - The XRD pattern of the MTZ.B–capped copper NPs using (copper (II) sulphate salt solution, pvp, ascorbic acid and MTZ.B (0.2 , 10.8 x 10⁻³ , 0.289 and 3.6 x 10⁻⁴ M respectively) at pH 7
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Atomic force microscopy (AFM) analysis
The AFM analysis for copper nanoparticles, Figure (7) shows that the heterogeneously-shaped copper nanoparticles were of average particle diameters around 47.20 nm.

Figure 7- AFM picture and granularity cumulation distribution chart of copper nanoparticles with average size diameter around 47.20 nm.

The AFM analysis for MTZ.B -capped copper nanoparticles Figure (8) shows that copper nanoparticles have uniform or regular shapes with average diameter of 73.56 nm.
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Figure 8 - AFM picture and granularity cumulation distribution chart of (MTZ.B) capped copper nanoparticles with average size diameter around 73.56 nm, using (copper (II) sulphate solution), pvp, ascorbic acid and MTZ.B (0.2 , 10.8 x 10⁻³ , 0.289 and 3.6 x 10⁻⁴ M respectively) at pH 7.

FT-IR spectrophotometry
The FT-I R spectra of MTZ.B, L- ascorbic acid, Cu-NPs, MTZ.B-capped Cu NPs are shown in Figure (9). Infrared spectrum of pure metronidazole Benzoate are shown in Figure (9 MTZ.B) exhibited a band at around 3419.56 cm⁻¹ is attributed to aromatic C-H stretching [26, 27]. The band at around 1452.30 cm⁻¹ is C- C ring strectcing vibration [27], while the band assigned to (out of plane) ring C=C bending was located at 689.35 cm⁻¹[26, 27]. The band at around 3373.27 cm⁻¹ to C-H (olefin) stretching vibration of imidazole ring [ 20, 26, 28], while the band assigned to bending vibration (out of plane) of this bond was located at 709.76 cm⁻¹[28]. The band appeared at 2985.74cm⁻¹ is due to aliphatic C-H stretching vibration [26, 27, 28]. The band observed at 1427.23 cm⁻¹ was due to C=C stretching vibration [26], while band observed at 825.23cm⁻¹ to C-C (alkanes) stretching vibration [20, 28].
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The N-O asymmetrical and symmetrical stretching vibrations of NO\textsubscript{2} group was located at 1523.66 cm\textsuperscript{-1} and 1355.86 cm\textsuperscript{-1} respectively[20, 26, 28]. Band observed at 1465.80 cm\textsuperscript{-1} is attributed to C =N stretching vibration of imidazole ring [26]. The band at around 1188.07 cm\textsuperscript{-1} to C - N (tertiary amine group) stretching vibration [20, 28]. The band at around 1716.53 cm\textsuperscript{-1}was attributed to C=O group stretching vibration [17, 18, 26, 27], the band at around 1070.42 cm\textsuperscript{-1} to C-O group stretching vibration [27].

Infrared spectrum of L- ascorbic acid is shown Figur 9. The spectrum exhibited "four strong IR bands in the region 3213.19 cm\textsuperscript{-1}, 3313.48, 3409.91 and 3525.63 cm\textsuperscript{-1}" assigned to OH stretching vibration [17, 18, 26]. The band at around 3028.03 cm\textsuperscript{-1} was due to - CH\textsubscript{2} group stretching vibration [17, 18]. The band at around 1753.17 cm\textsuperscript{-1}was attributed to C=O group stretching vibration [17, 18, 26, 27]. The bands observed at around 1456.16 cm\textsuperscript{-1} was due to C=C group stretching vibration [17, 18, 26], while the band appeared at 1072.35 cm\textsuperscript{-1} was attributed to C-O stretching vibration [18, 27].

Figure (9 Cu NPs) shows FT- IR spectra of copper NPs prepared the reaction mixture (copper (II) sulphate salt solution, pvp, and ascorbic acid) (0.2 M, 10.8 x \(10^{-3}\) M, and 0.289M, respectively). The spectrum of copper NPs exhibited the shift of the band at 3028.03 cm\textsuperscript{-1} assigned to - CH\textsubscript{2} group stretching vibration [17, 18] in the spectrum of free L-ascorbic to 3049.25 cm\textsuperscript{-1}, and the disappearance of the peaks at 3525.63 and 3213.19 cm\textsuperscript{-1} assigned to OH stretching vibrations of L- ascorbic acid [17, 18, 26] and the bands observed at around 1456.16 cm\textsuperscript{-1} attributed to C=C group stretching vibration [17, 18, 26] with the appearance of low absorption peaks at 1747.39 cm\textsuperscript{-1} and 1683.74 cm\textsuperscript{-1} stretching vibration of carbonyl groups. Band at 1072.35 cm\textsuperscript{-1} which was attributed to C-O stretching vibration [17, 18] in the spectrum of L- ascorbic acid was shifted to lower wave number and appeared at 1066.56 cm\textsuperscript{-1}. These results refer to the oxidation of ascorbic acid as a result of Cu NPs synthesis [4, 12].

The spectrum of the MTZ.B-capped Cu-NPs is shown in Figure ( 9 MTZ.B- Cu NPs) exhibited the band attributed to aromatic C-H stretching [26, 27] in the spectrum of MTZ.B was shifted to the higher wavenumber and appeared at 3444.76 cm\textsuperscript{-1}.The band attributed to C-C ring stretching vibration [27] was shifted to the lower wavenumber and appeared at 1431.08 cm\textsuperscript{-1}. The band assigned to (out of plane) ring C=C bending [26, 27] was shifted to the lower wavenumber and appeared at 680.83 cm\textsuperscript{-1}. The band assigned to C-H (olefin) stretching vibration of imidazole ring [ 20, 26, 28] was shifted to the lower wavenumber and appeared at 3224.76 cm\textsuperscript{-1},
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band asigned to aliphatic C-H stretching vibration [26, 27, 28] was shifted to the higher wavenumber and appeared at 3066.61 cm\(^{-1}\), band assigned to C-C (alkanes) stretching vibration [20, 28] shifted to the higher wavenumber and appeared at 864.05 cm\(^{-1}\) in the spectrum of MTZ.B-capped Cu-NPs.

The N-O asymmetrical and symmetrical stretching vibrations of NO\(_2\) group [20, 26, 28] in the spectrum of MTZ.B were shifted to the wavenumber at 1537.16 and 1371.29 cm\(^{-1}\) respectively in the spectrum of MTZ.B-capped Cu-NPs. The band attributed to C=N stretching vibrations [26] shifted to 1479.30 cm\(^{-1}\). The bands assigned to the stretching vibrations of C-N (tertiary amine) [20, 28] and C-O bonds [27] were shifted to the higher wavenumbers and appeared at 1189.07 and 1076.21 cm\(^{-1}\) respectively.

The spectrum of MZ / Cu NPs exhibited also the disappearance of the two sharp peaks which were observed at 3525.63 and 3313.48 cm\(^{-1}\) assigned to OH stretching vibrations of L-ascorbic acid and appearance of a new weak band at 1672.02 cm\(^{-1}\) which confirms the oxidation of L-ascorbic acid by Cu (II) ions as a result of Cu NPs synthesis [4, 12] by which C-OH was converted to carbonyl group. The band assigned to C=O stretching vibration of the free AA was shifted to higher wave number and appeared as a very weak band at 1782.23 cm\(^{-1}\). These results indicate that Cu NPs have been capped by both ascorbic acid and MTZ.B molecules.
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Figure 11- The FT-IR spectrum of MTZ.B, L- ascorbic acid, Cu NPs and MTZ.B capped Cu NPs

Conclusions

Cu NPs with different particle sizes synthesized via reduction of Copper (II) sulfate pentahydrate salt (Cu SO₄.5H₂O) using ascorbic acid as a reducing agent and polyvinyl pyrrolidone PVP (M.Wt 10,000) as a stabilizing agent in presence of Metronidazole Benzoate drug. The average diameters in particle size distribution charts by the AFM image were around 47.20 nm and 73.56 nm. The FTIR spectra of the prepared Cu NPs showed that the ligand molecules have been covalently attached on the surface of Cu NPs.

References:
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تحضير وتشخيص دقائق النحاسة النانوية بطريقة كيميائية باستخدام حامض الاسكوربيك كعامل مختزل بوجود دواء ميترونيدازول بنزويت (1- (2- بينزيل اوكسي اثيل)-5-نايترتو ميثيل اميدازول)

مي جودة كريم

العراق , بغداد

الخلاصة

تم تحضير دقائق النحاسة النانوية بطريقة كيميائية باستخدام كبريتات النحاس المائية

عامل استقرارية مع pvp (Bose من حامض الاسكوربيك كعامل مختزل و CuSO4 . 5H2O)

دواء ميترونيدازول بنزويت بطريقة كيميائية. شحنت المذابات النانوية الناتجة بواسطة مطيافية

الإشعاع فوق البنفسجي- المرئية وتحاليل مجهر القوى الذرية, وحيود الإشعة السينية ومطيافية

FT-IR-